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RESEARCH ARTICLE

Determination of metals and BTEX in different components of waterpipe: charcoal, tobacco, smoke and water

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Abstract
Background The main objective of this study was to evaluate the concentrations of heavy metals and BTEX (benzene, toluene, ethylbenzene and xylene) in smoke and water bowl of 5-most commonly used tobacco brand in waterpipe in Tehran, the capital of Iran.
Methods Five types of conventional tobacco in Tehran were investigated. Heavy metals and BTEX were analyzed in waterpipe smoke, tobacco, charcoal and water bowl prior to and after smoking by using ICP-OES and GC-MS, respectively.
Results Our results indicated that Khansar and Al Fakher brands had the maximum and minimum concentrations of metals among tobacco consumed, respectively. The results showed that there was a significant difference between content of heavy metals in burned and unburned tobacco. The highest and lowest concentrations of metals were related to Fe and Hg, respectively.
Conclusion Results showed that tobacco, charcoal and smoke of waterpipe contained significant contents of toxic metals and BTEX, and exposure to these components could be the main reason for the concerns about waterpipe smoking.

Keywords Waterpipe · Tobacco · Smoke · Heavy metals · BTEX

Background

Tobacco smoking is a preventable cause of death throughout the world. Most of the cigarette smokers believe by mistaken that waterpipe smoking is a fun social activity, which leads to more social behavior and comfort that is safer or less dangerous for health than cigarette smoking [1–4]. In fact, waterpipe smokers are exposed to hundreds of toxic substances (such as heavy metals) [5], many of which are definitely carcinogenic for humans [6]. Waterpipe smoking has been reported abundantly in high-, middle-, and low-income countries. In high-income countries, death rate attributed to tobacco smoking will be reduced by 9% from 2002 to 2030, but in low- and middle-income countries, it will be doubled and the number of deaths caused by tobacco smoking is estimated to be 3.4 to 6.8 million [7]. Recently, waterpipe smoking has significantly increased in the southwestern Asia and northern Africa, especially among the youth [8, 9]. Nowadays, waterpipe smoking is unfortunately prevalent among university as well as high school students [10]. The prevalence of waterpipe smoking is 100 million per day in the world and its popularity is rising among the youth [11]. In the study performed in 2010 in the United States, among high school students, 1 out of 5 boys (about 17%) and 1 out of almost 6 girls (about 15%) have smoked waterpipe at least once during the previous year [12]. The findings from other studies that have been carried out on young adults indicate high prevalence of waterpipe smoking among university students in the United States and this has been also reported within 22–40% [9, 13].

Studies have shown that the waterpipe smoker inhales 50 to 100 l smoke in 45 min, containing large amounts of toxic and carcinogenic agents [14]. Results of numerous studies that have examined the amount of substances in tobacco and waterpipe smoke have shown different toxic agents including

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carbon monoxide, hazardous compounds such as volatile organic compounds (VOCs), and heavy metals in the waterpipe smoke [15–19]. Due to the inhalation of a large amount of smoke and prolonged exposure to smoke, waterpipe smoking increases the probability of adverse effects on human health. Research has shown that the level of exposure to toxic and carcinogenic compounds caused by waterpipe smoking is equal to/or greater than exposure to such compounds caused by cigarette smoking [14, 20]. Besides, research carried out on waterpipe smokers shows higher ratio of chronic bronchitis than cigarette smokers [21]. Tobacco smoking causes many acute and chronic diseases, including cardiovascular diseases, chronic obstructive pulmonary diseases, and lung cancer [22]. The most important pollutants in waterpipe smoke that cause several health effects are heavy metals and VOCs. Tobacco is a rich source of heavy and toxic metals, the amount of which is increased during plant growth. In the study by Verma and et al. on the prevalent types of tobacco of different brands in India, the level of 7 heavy metals was examined and specified. Results showed that the amount of these metals was high and differed across tobacco products and brands [23]. Moreover, some studies showed that about 40–60% of the cadmium inhaled through the tobacco smoke directly enters the blood [24]. Benzene, ethylbenzene, toluene, and xylenes, known collectively as BTEX (benzene, toluene, ethylbenzene and xylene), represent a significant fraction of the volatile organic compounds emitted in waterpipe smoke [5]. Benzene, a tobacco and charcoal toxic constituent, has been quantified in cigarette and hookah tobacco first-hand smoke, and has been assessed as the predominant aromatic compound emitted from glowing charcoal [25]. The International Agency for Research on Cancer (IARC) has classified benzene as an intense carcinogenic agent and ethylbenzene as a suspected carcinogenic compound (IARC, 1999).

Iran is one of the earliest areas, in which waterpipe smoking has been reported, with fairly high prevalence of waterpipe smoking [16]. Therefore, with respect to the importance of this risk factor and since no study has been conducted so far on heavy metals and BTEX contents in waterpipe first-hand smoke, this study determined the concentration of heavy metals and BTEX in 5 most commonly used tobacco brands in Tehran, the capital of Iran. Indeed, several studies have been conducted in Iran on the amount of heavy metals in tobacco [16, 26] and BTEX in waterpipe cafés or traditional restaurants [27, 28]. A study carried out in 2017 to investigate the concentration of heavy metals in two most widely used tobacco brands and waterpipe water in coffeeshops in city of Sanandaj, Iran showed that the highest and lowest concentrations of metals were related to zinc and arsenic, respectively [16]. Also, based on the results of a study that was performed in 2012, the maximum amount of heavy metals in the widely used cigarettes and flavoured tobaccos (maassel) in the Isfahan, Iran market had been Fe > Zn > Ni > Cu > Cd > Pb > Cr, respectively [26].

The main objective of this study was to evaluate the concentration of heavy metals and BTEX in first-hand smoke, tobacco, charcoal and water bowl prior to and after smoking of 5-most commonly used tobacco brand in waterpipe in Tehran, Iran.

Methods

Study design

This study was performed in the laboratory conditions and as a pilot (Fig. 1). In order to simulate real conditions, a conventional type of waterpipe in Tehran with metal body, glass water bowl and plastic hose with the length of 150 cm made of polyurethane was used. To create suction, a suction pump (Vacuum Pump Sparmax TC-501 N) with flow of 12 L min^{-1} was used. In this work, the method proposed by Shihadeh (2004) was employed for the pilot implementation (Shihadeh, 2004). Briefely, in this study every waterpipe session included 171 puffs, every puff lasted for 2.6 s, volume of every puff was about 530 ml and the interval between the suctions was almost 20 s. Moreover, in every session, the waterpipe bowl was filled with 750 ml distilled water, so that 30 mm of the body was placed under water. Before every time of pilot launching, 10 g tobacco was weighed by a digital scale and, then, it was placed on top of the waterpipe. Top of the waterpipe was covered with an aluminum foil (with diameter of 9 cm) which was perforated (18 holes), so that tobacco does not touch the aluminum sheet. Afterwards, charcoal with the weight of 6 g was placed on the oven for 60 s and the heated coal was placed on top of the perforated foil. In this study, 5 types of conventional tobacco in Tehran city, namely Al Mahmood, Al Fakher, Bahreini, Nakhla (flavored tobaccos or maassel) and Khansar (traditional tobacco), were examined.

Analysis of metals

In order to determine the concentration of metals in the waterpipe first-hand smoke, a filter holder was installed at the inlet to the pump and after the waterpipe outlet interface and a fiberglass filter was placed in the inside; the waterpipe smoke passed through the pump after passing through the filter and collecting the particles. After every session, the filters were maintained inside the Petri dish and, until the time extraction and analysis, they were kept in the refrigerator at −20 °C. In this work, the extraction of metals was performed by single reaction chamber microwave digestion System (Milestone, Italy). To prepare for digestion, 2.0 ± 0.05 g of the homogenized sample was accurately weighed in a PTFE

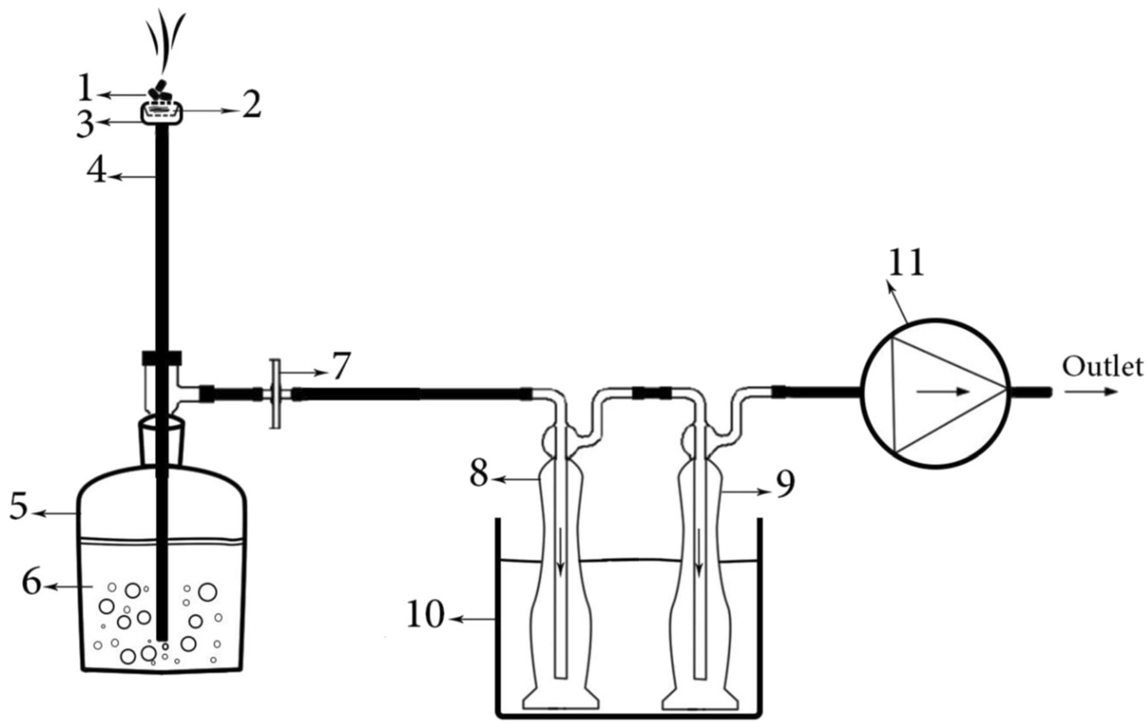

Fig. 1 Experimental set-up for determination of metals and BTEX in different components of waterpipe: charcoal (1), tobacco (2), head (3), stem (4), bowl (5), water (6), filter holder (7), impingers (8 and 9), cold bath (dry ice / isopropyl alcohol) (10), pump (11)

digestion vessel, and then 10 ml of nitric acid (68%, (*v*/v)), was added. The vessels were closed and placed inside the microwave digestion system. After digestion, the volume was finally made up to 25 ml using ultrapure water [8]. The extracted solution was passed through the syringe filter with the pore size of 0.45 μm and, then, analyzed using ICP-OES instrument (Perkin Elmer 8000, USA). In this study, the content of metals in the burned and unburned tobacco and charcoal, smoke emitted from waterpipe and water in the waterpipe bowl was determined.

Analysis of BTEX

To determine BTEX in the waterpipe smoke, 2 impingers (100 ml), each containing 20 ml methanol, were connected to the waterpipe outlet and the impingers were placed inside the cold bath containing dry ice and isopropyl alcohol [22]. As shown in Fig. 1, smoke emitted from the waterpipe outlet was passed through 2 impingers connected to each other in series. After each waterpipe session and for BTEX identification, the contents of each impinger were severely shaken and, then, 1 ml was taken from each impinger [22]. Afterwards, the containing compounds were identified by GC-MS [28]. The GC-MS analysis was performed on an HP 7890B gas chromatograph equipped with an Agilent MSD 5977B mass spectrometer (Agilent Technologies, Waldbronn, US) and a Cold Injection System (CIS) from Gerstel (Gerstel, Mühlheim an der Ruhr, Germany).

Quality assurance and quality control

For Quality Assurance and Quality Control (QA/QC), laboratory blanks as well as spiked samples were analyzed along with the samples. Recovery efficiencies, determined as the averages of extractions of spiked samples, ranged from 85 to 108% for metals, and from 91 to 104% for the BTEX. The limits of detection (LoD) were set as three times the standard deviation of the blank values. The average of blank values was then subtracted from all concentrations above the LoD values.

Data analysis

Data were statistically processed using SPSS ver. 22 (IBM Corp.,USA). Descriptive statistics were applied for the presentation of the results. The paired-sample t-test was used to determine the statistical significance ($P < 0.05$) of the difference between the metal contents of burned and unburned and tobacco type.

Results and discussion

In this study, the content of metals in the first-hand smoke, waterpipe water, tobacco, and charcoal was investigated.

Table 1 Metals concentration in waterpipe unburned and burned tobacco types

Metals	Tobacco types (Average ± SD) ($\mu g\ g^{-1}$)									
	Al fakher		Nakhla		Al mahmood		Khansar		Bahreini	
	Unburned	Burned	Unburned	Burned	Unburned	Burned	Unburned	Burned	Unburned	Burned
Mn	2.92 ± 1.2	4.71 ± 1.88	4.09 ± 1.63	2.16 ± 0.86	0.85 ± 0.21	1.73 ± 0.69	12.91 ± 5.16	16.57 ± 6.62	10.76 ± 4.30	15.39 ± 6.15
Cr	0.29 ± 0.1	0.48 ± 0.25	0.43 ± 0.23	1.00 ± 0.32	0.31 ± 0.10	0.67 ± 0.24	3.56 ± 1.20	4.55 ± 1.60	ND	ND
Cu	1.34 ± 0.44	1.51 ± 0.50	1.60 ± 0.53	1.84 ± 0.61	1.22 ± 0.40	1.65 ± 0.55	4.14 ± 1.38	4.79 ± 1.59	1.83 ± 0.61	1.91 ± 0.63
Ni	0.26 ± 0.12	0.14 ± 0.10	ND	0.21 ± 0.13	0.36 ± 0.20	1.76 ± 0.43	0.51 ± 0.21	1.68 ± 0.47	2.32 ± 0.73	ND
Al	6.15 ± 2.19	8.53 ± 3.04	21.44 ± 7.65	23.40 ± 8.35	8.71 ± 3.11	15.66 ± 5.59	146.06 ± 52.16	199.02 ± 71.07	24.76 ± 8.84	21.45 ± 7.66
Pb	2.04 ± 0.66	2.18 ± 0.42	1.44 ± 0.58	0.83 ± 0.30	0.55 ± 0.11	0.83 ± 0.36	0.31 ± 0.20	ND	ND	ND
Fe	6.40 ± 2.66	16.79 ± 6.99	16.98 ± 7.07	27.01 ± 11.25	10.24 ± 4.26	26.46 ± 11.02	139.44 ± 58.1	189.49 ± 78.95	22.78 ± 9.49	23.51 ± 9.79
Co	0.07 ± 0.02	0.06 ± 0.01	0.08 ± 0.03	0.01 ± 0.01	0.08 ± 0.02	0.00	0.15 ± 0.06	ND	ND	ND
Sn	7.59 ± 2.44	9.55 ± 3.08	11.56 ± 3.72	14.75 ± 4.75	10.14 ± 3.27	15.35 ± 4.95	60.88 ± 19.63	70.49 ± ±22.73	10.34 ± 3.33	10.10 ± 3.25
Zn	1.25 ± 0.48	1.94 ± 0.74	1.50 ± 0.57	2.11 ± 0.81	1.01 ± 0.38	1.89 ± 0.72	6.27 ± 2.41	6.13 ± 2.35	4.31 ± 1.65	5.50 ± 2.11
Cd	0.16 ± 0.04	0.16 ± 0.03	0.13 ± 0.09	0.22 ± 0.09	ND	0.04 ± 0.01	ND	0.21 ± 0.07	ND	ND
Mo	ND	ND	0.03 ± 0.01	0.21 ± 0.07	0.36 ± 0.07	ND	ND	ND	ND	ND
Sb	ND	2.34 ± 0.94	2.06 ± 0.82	0.93 ± 0.6 2	0.53 ± 0.42	2.44 ± 1.00	1.99 ± 0.95	2.18 ± 1.08	ND	ND
As	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hg	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND Not Detected

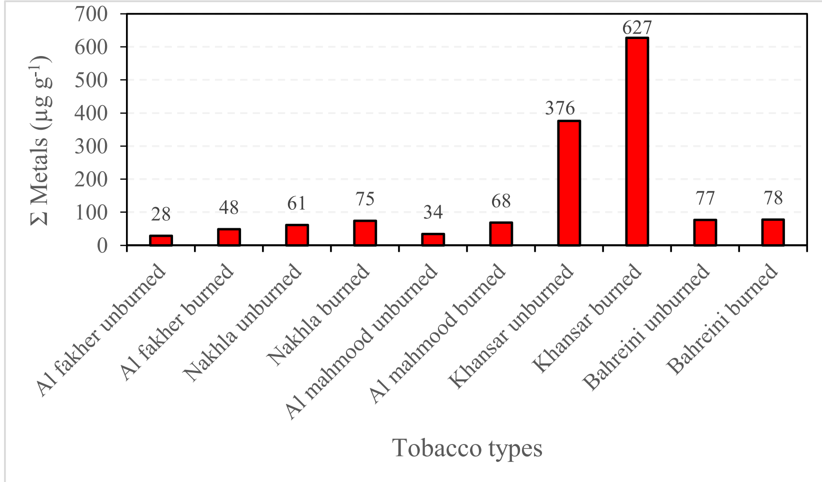

Fig. 2 The concentration of the sum of metals in burned and unburned tobacco types

Metals in burned and unburned tobaccos

Results of the heavy metals content of five burned and unburned tobacco brands are presented in Table 1.

As shown in Table 1, the mass rate metals such as *Zn, Sn, Fe, Al, Cu,* and *Mn* was detected in all the cases, while *As* and *Hg* were not detected in any of the samples.

The results presented in Fig. 2 indicate that the content of metals in burned tobacco was higher than that in the unburned one per unit of mass ($P < 0.05$). This may be due to metal concentrated process in burnt tobacco. Also, the ratio of sum of metals in the unburned to burned tobacco was equal to 2 in Al Mahmood Tobacco, which was maximum among other brands. However, the minimum was found as 1 in Bahrain Tobacco. Values of this ratio in Khansar and Al Fakher Tobacco were similar and equal to 1.7, while it was about 1.2 in Nakhla Tobacco. As shown in Fig. 2, the sum of metals per gram in Khansar Tobacco was approximately 13 times more than that in Al Fakher Tobacco. Also, the content of metals per gram of Khansar Tobacco (376 μg g^{-1}) was higher than the sum of all the unburned tobacco products (200 μg g^{-1}). Moreover, the maximum and minimum sum of metals were found in Khansar (burned: 627 μg g^{-1} and unburned: 376 μg g^{-1}) and Al Fakher Tobacco (burned: 48 μg g^{-1} and unburned: 28 μg g^{-1}), respectively.

In Fig. 3, share percent of every metal in the total content of metals is given.

As shown in Fig. 3, *Fe* and *Al* had maximum content of total metals in all types of tobacco. Then, *Sn*, *Mn,* and *Zn* had the maximum percent of total metals contents, the results of which were consistent with those of Elsayed's study [29]. The maximum concentration of *Zn* was observed in the Khansar sample. On the other hand, *Co* and *Sb* were available in relatively low concentrations in all the tobacco samples.

The IARC introduced *Cd, Cr, Ni*, and *As* metals as definitive carcinogenic for humans. In Fig. 4, results of the contents of carcinogenic metals in various types of tobacco are presented.

Results of this study showed that the total content of carcinogenic metals in 5 tobacco brands in Tehran ranged from 0 to 4.5, which formed 20–30% of the total content of metals. The results indicated that the maximum content of the carcinogenic heavy metals in each of the 5 tobacco brands examined was as follows: $Cr > Ni > Cd$. Among the carcinogenic metals, *Ni* and *Cr* had higher concentrations in Bahreini and Khansar brands than other metals, respectively. The high level of *Cd* in the results could be justified by its recognized accumulation

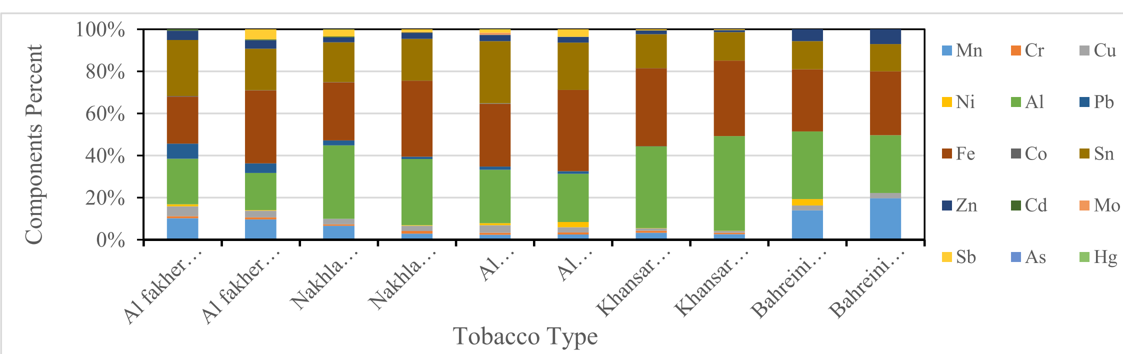

Fig. 3 Percent of the components of metals existing in various tobacco types

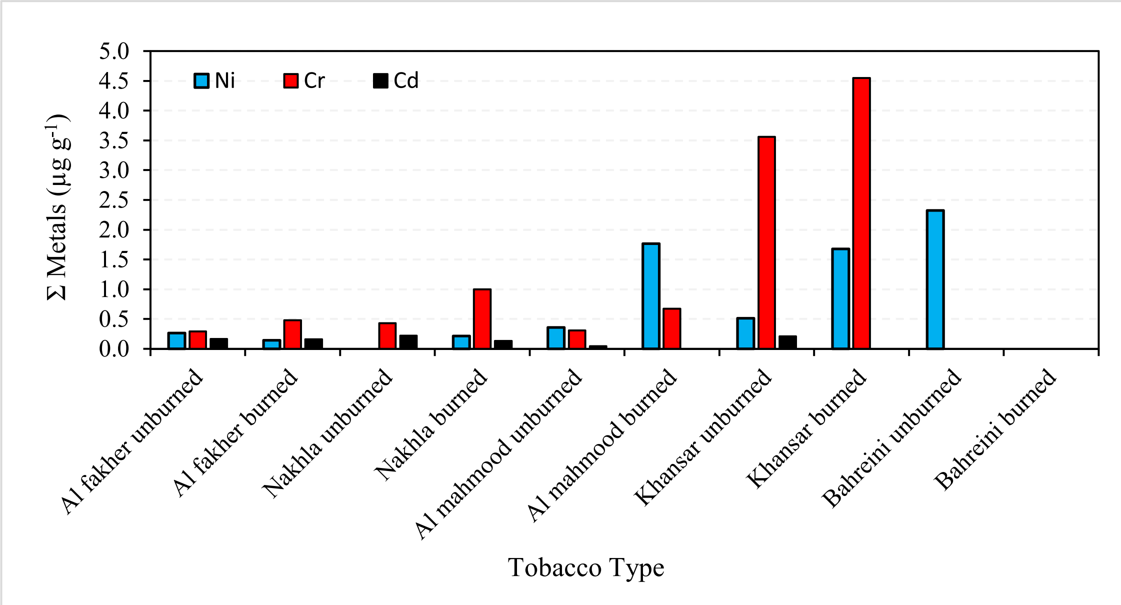

Fig. 4 Concentration of carcinogenic metals in different tobacco types

in the tobacco plant [28]. According to Fig. 3, the highest content of heavy metals was found in Khansar Tobacco ($P < 0.05$). In a study on 617 tobacco samples, the mean concentrations of *Pb* and *As* were 0.93 and 0.15 μg g^{-1}, respectively [30].

Metals in charcoal and water bowl of waterpipe

In Table 2, the content of metals in water bowl and burned and unburned charcoal are presented.

Results of Table 2 show that the concentration of metals in the burned charcoal was significantly increased compared to the burned one ($P < 0.05$). The content of *Fe*, *Al*, and *Mn* had the highest value in the burned charcoal. Also, the content of carcinogenic metals found in the burned charcoal was high ($Ni > Cd > Cr > As$). In charcoal-induced smoke, there are compounds that could cause eye, skin, respiratory tract, and gastrointestinal tract irritation [29]. Moreover, the role of charcoal as a significant source of toxicity (source of *PAHs* and *Co*) in waterpipe was emphasized [31].

Table 2 The concentration of metals in waterpipe charcol and water bowl before and after smoking (Average ± SD)

Metals	Charcoal (μg g^{-1})		Water bowl (μg L^{-1})	
	Unburned	Burned	Before smoking	After smoking
Mn	30.54 ± 11.31	176.74 ± 65.45	29.10 ± 10.77	115.15 ± 42.64
Cr	3.08 ± 1.02	26.41 ± 8.80	ND	33.17 ± 11.05
Cu	1.92 ± 0.73	17.15 ± 6.59	156.71 ± 60.27	182.70 ± 70.26
Ni	0.38 ± 0.20	76.26 ± 40.13	6.44 ± 3.38	21.00 ± 11.05
Al	37.27 ± 10.07	448.65 ± 121.25	56.21 ± 15.19	263.20 ± 71.13
Pb	ND	8.01 ± 4.45	ND	296.06 ± 164.47
Fe	124.83 ± 31.20	1045.81 ± 261.45	73.31 ± 18.32	335.12 ± 83.78
Co	0.20 ± 0.08	0.55 ± 0.22	ND	2.76 ± 1.10
Sn	57.45 ± 16.89	458.50 ± 134.85	19.29 ± 5.67	595.80 ± 175.23
Zn	5.25 ± 1.45	50.36 ± 13.98	165.00 ± 45.83	361.46 ± 100.40
Cd	0.22 ± 0.13	0.54 ± 0.25	ND	4.24 ± 2.12
Mo	ND	ND	0.97 ± 0.60	50.18 ± 31.46
Sb	2.81 ± 1.42	13.20 ± 6.80	ND	53.01 ± 26.51
As	0.01 ± 0.006	ND	ND	ND
Hg	0.08 ± 0.05	0.05 ± 0.03	ND	1.80 ± 1.13

ND Not Detected

Table 3 The concentration of metals in the waterpipe first-hand smoke

Metals	Tobacco type (Average ± SD) (μg m^{-3})				
	Al fakher	Nakhla	Al mahmood	Bahreini	Khansar
Mn	1.42 ± 0.94	1.49 ± 0.99	1.54 ± 1.03	1.71 ± 1.14	1.78 ± 1.18
Cr	2.34 ± 1.8	2.78 ± 2.14	1.81 ± 1.42	2.50 ± 1.92	3.37 ± 2.61
Cu	3.87 ± 1.48	3.98 ± 1.53	2.51 ± 0.96	3.67 ± 1.42	4.95 ± 1.90
Ni	38.94 ± 12.98	10.48 ± 3.50	7.29 ± 2.43	30.30 ± 10.44	23.83 ± 7.94
Al	77.31 ± 26.65	77.05 ± 25.57	79.88 ± 27.54	92.47 ± 31.88	120.16 ± 41.43
Pb	13.12 ± 7.71	22.18 ± 13.04	16.85 ± 9.91	21.70 ± 12.75	21.91 ± 12.88
Fe	25.53 ± 12.15	17.50 ± 8.33	16.36 ± 7.79	29.58 ± 14.08	58.48 ± 27.84
Co	0.80 ± 0.57	0.98 ± 0.70	0.31 ± 0.22	0.64 ± 0.45	0.74 ± 0.52
Sn	28.76 ± 7.19	27.62 ± 6.73	19.03 ± 4.64	31.12 ± 7.59	43.29 ± 10.55
Zn	164.96 ± 35.09	180.86 ± 38.48	168.70 ± 35.91	186.27 ± 39.63	245.75 ± 52.27
Cd	0.44 ± 0.14	0.63 ± 0.20	0.32 ± 0.11	0.19 ± 0.06	0.65 ± 0.20
Mo	2.77 ± 1.34	3.07 ± 1.09	0.10 ± 0.03	1.98 ± 0.70	0.11 ± 0.04
V	0.71 ± 0.37	2.45 ± 1.29	1.25 ± 0.65	2.31 ± 1.23	2.35 ± 1.20
Sb	1.91 ± 1.06	1.13 ± 0.62	0.13 ± 0.06	ND	ND
As	ND	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.05 ± 0.02
Hg	ND	ND	ND	ND	ND

ND Not Detected

In the waterpipe water bowl, *Pb* and *Cd* and a small amount of *As* metals were identified [16]. The compared results of heavy metals before and after the use of waterpipe in the waterpipe water bowl showed a significant difference in metals, and the average concentration of metals in the waterpipe water bowl increased.

Metals in the waterpipe first-hand smoke

Table 3 shows the content of metals in the waterpipe first-hand smoke of various types of tobacco.

As can be observed, the contents of *Al*, *Zn*, and *Ni* metals were very high in the waterpipe first-hand smoke. It is worth noting that tobacco and charcoal were not the only potential sources of exposure to these metals. For example, aluminum foil or waterpipe body can also release these metals [22]. Accordingly, an increasing content of lead in waterpipe first-hand smoke was also due to the charcoal burning.

BTEX in the waterpipe first-hand smoke

In Table 4, the BTEX found in the waterpipe first-hand smoke of different types of tobacco are presented.

Results of the present study showed that waterpipe first-hand smoke contained BTEX and pyridine. According to conducted studies, waterpipe smoking can increase the possibility of adverse effects of these compounds on the human health. IARC has introduced benzene as a definite carcinogenic compound for humans. According to previous works, chronic exposure to benzene could lead to damage to bone marrow blood cells and increase the risk of developing leukemia in men. Also, human exposure to BTEX compounds might cause neurological disorders and symptoms such as weakness, loss of appetite, fatigue, confusion, and nausea [32].

Results of the studies show that, by charcoal burning, high levels of benzene and less content of toluene are produced. Charcoal is the main source of benzene. By heating up the tobacco, isoprene and pyridine are also produced [22, 29,

Table 4 BTEX levels in the mainstream waterpipe first-hand smoke

BTEX	Waterpipe first-hand smoke (μg/session)					
	Al fakher	Nakhla	Al mahmood	Bahrini	Khansar	Other Studies
Benzene	280	275	300	290	13	271 [22]
Toluene	12	11	14	10	8	9.92 [22]
Ethylbenzene	1.3	1.6	1.5	1.9	1	1.00 [22]
p-Xylene	–	–	–	–	–	0.929 [22]
m-Xylene	–	–	–	–	–	2.47 [22]

33]. A high concentration of benzene is reported in the main flow of waterpipe, which is 6.2 times more than that of first-hand smoke [22]. Also, people who use flavored tobacco are exposed to higher concentrations of BTEX compounds, resulting in the increased cancer and chronic non-cancerous risks [28].

Based on the studies on waterpipe first-hand smoke, there are higher levels of metals (such as Ni, Co,Cr, Pb and As) CO, Tar, nicotine, PAHs and aldehydes than in cigarette [8, 31]. Biomarkers exposed to these chemicals are measured at a significant level among waterpipe consumers [33]. An experimental clinical study showed that people who smoked waterpipe were exposed to carcinogenic compounds of waterpipe first-hand smoke and their risk of developing cancer and other chronic diseases increase. In this study, the content of nicotine and mercapturic acid metabolites of the volatile organic compounds in people's urine were increased after waterpipe smoking by 73 times and 14–91%, respectively [34].

Conclusions

In summary, results showed that tobacco and charcoal contained significant contents of toxic metals and exposure to these elements could be the main reason for the concerns about waterpipe smoking. Our results indicated that Khansar and Al Fakher brands had the maximum and minimum concentrations of metals among tobacco consumed, respectively. The toxic metals and hazardous organic compounds existing in the waterpipe first-hand smoke indicated that long-term exposure to it can have adverse effects on the human health.

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Compliance with ethical standards

Ethics approval and consent to participate Not applicable.

Conflict of interest The authors declare that they have no conflict of interest.

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